duced more MMP-1 or MMP-1 after IL-1α or TNF-α stimulation as compared to the hip chondrocytes that were derived from the same animal.
Conclusions: MMP-1 and MMP-13 have been implicated in type II collagen degradation in cartilage. We have shown for the first time that MMP-1 and MMP-13 production differs in knee and hip cartilage at different ages in porcine chondrocytes. Chondrocytes from newborn pigs produced the most collagenases after IL-1α or TNF-α stimulation. This may be due to high rate of ECM turnover needed for skeletal growth, as oppose to young adult pigs. Interestingly, this responsiveness reoccurs in the chondrocytes from old adult pigs and may result in ECM remodeling in joints of older individuals. Further study into the signaling of IL-1α and TNF-α on MMP-1 and MMP-13 production in aging knee and hip cartilage could provide different pathways in collagenase synthesis in aging joints.

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P96

MURINE EPIPHYSEAL CHONDROCYTE AGGREGATES EXHIBIT SPONTANEOUS ADAMTS-MEDIATED AGGRECAN CLEAVAGE IN VITRO

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This study assessed ADAMTS "aggrecanase" expression and activity in murine neonatal epiphyseal chondrocytes, maintained as non-adherent aggregates in vitro.
Distal femoral and proximal humeral epiphyses were collected from 4-day-old mice. Chondrocytes were isolated by collagenase digestion and cultured as non-adherent aggregates in serum-free medium supplemented with ascorbic acid. Cultures were maintained for up to 12 days, with sample collections at 3-day intervals. GAG content in the pericellular and medium compartments was monitored by DMMB assays. Aggrecan expression and degradation were assessed by Western blot analyses of chondroitinase-treated lysates, using antibodies specific for the full-length protein and neoepitopes created by ADAMTS activity. Expression of cartilage-linked ADAMTS genes (TSs 1, 4, 5, 8, 9 and 15) was determined by RT-PCR. Immunolocalization of ADAMTS 4 and 5, the NITEGE neo-epitope and hyaluronan was carried out using confocal microscopy to determine the spatial interactions between these targets in the three-dimensional context of the aggregates.
Murine epiphyseal chondrocytes synthesized 60-80 μg GAG/million cells/3-days. Approximately 10% of total GAG was retained in the pericellular compartment while 90% was released into the medium. Aggrecan immunoblotting demonstrated spontaneous degradation of the full-length protein with neoepitope expression characteristic of ADAMTS activity. Epiphyseal chondrocytes expressed all six of the cartilage-linked ADAMTSs, as assessed by RT-PCR. Confocal immunolocalization of ADAMTS 4 and 5 in the aggregates demonstrated that ADAMTS4 is restricted to the surface cells only, whereas -TS5 is distributed throughout the aggregates. These data suggest that ADAMTS expression is influenced by spatial or intercellular cues. Further, the NITEGE neo-epitope, that signifies ADAMTS cleavage of the aggrecan core protein, was excluded from regions that stained positively for hyaluronan. Hyaluronan interactions with the aggrecan complex might confer resistance to ADAMTS proteolytic activity in this model.
The neonatal murine epiphyseal chondrocyte model is a valuable tool for investigating aggrecan synthesis and turnover by ADAMTS proteases, since aggrecanase activity is an inherent property of the model, and does not require stimulation by retinoic acid or inflammatory cytokines. The murine epiphyseal model provides an excellent system to investigate pathways that regulate ADAMTS expression and activity through genetic manipulations of mouse strains and by pharmacological treatment of the culture model itself.

P97

FUNCTIONAL CHARACTERIZATION OF AN ORPHAN NUCLEAR RECEPTOR, REV-ERBAa (EAR1) IN CHONDROCYTE AND ITS POTENTIAL ROLE IN OSTEOARTHRITIS

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Aim of the Study: To evaluate the expression and function of Rev-ErbAa in cartilage and in osteoarthritis (OA).
Methods: The expression of Rev-ErbAa in cartilage was observed at both RNA and protein level in cartilage and chondrocytes derived from human and bovine donors by real time PCR and immunocytochemical techniques. The effect of cartilage catabolic agents like IL1 and TNF and anabolic agents such as IGF-1 and TGFb, on the expression of Rev-ErbAa was evaluated by real time PCR assay. The overexpression of Rev-ErbAa was achieved by either adenoviral transduction or treatment with a PPARa agonist whereas its expression was suppressed by use of antisense oligonucleotides. The effect of overexpression and suppression on the expression of genes encoding matrix degrading enzymes and production of Aggrecanase was measured by real time PCR and a chondrocyte-based Aggrecanase assay, respectively.
Results: An important feature of OA is the degradation of articular cartilage which is composed of matrix rich in type II Collagen and Aggrecan. This process is likely related to the excess synthesis and release of several catabolic factors such as proinflammatory cytokines, MMPs and Aggrecanases. Thus, the modulation of these catabolic factors may lead to the identification of new therapeutic targets for the treatment of OA in humans.
In a survey examining the expression of all 48 nuclear receptors, Rev-ErbAa, an orphan nuclear receptor, was found to be the most highly expressing nuclear receptor in chondrocytes. Unlike other liganded nuclear receptors Rev-ErbAa belongs to a subfamily of orphan receptors that are repressors of target gene transcription. Immunocytochemical assay revealed a restricted but prominent expression of Rev-ErbAa in the midzone of articular cartilage obtained from human donors. Treatment of isolated articular chondrocytes with known catabolic agents resulted in an induction of Rev-ErbAa, while stimulation with anabolic agents led to a decrease in its expression. Increased expression of Rev-ErbAa was associated with an increase in levels of mRNA encoding matrix degrading enzymes such MMP13 and ADAMTS-4 whereas a decrease in Rev-ErbAa expression led to a concomitant reduction in mRNA levels of matrix degrading enzymes and a corresponding decrease in IL-1-stimulated Aggrecanase enzyme production.
Conclusion: This is the first report demonstrating a key role of Rev-ErbAa in both catabolic and anabolic processes in cartilage. These data also suggest that modulation of Rev-ErbAa may be a novel means to regulate the expression and production of matrix degrading enzymes and that it represents a novel therapeutic target for OA.